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冠状病毒膜融合抑制剂的研究进展

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摘要 冠状病毒(CoVs)是普遍一类感染人和动物的包膜RNA病毒, 其特点是传播性强, 致病性高, 可引起人类和动物严重的临床症状及死亡。特异性药物的研发是控制冠状病毒感染的有效途径。膜融合过程是病毒进入靶细胞的关键步骤, 也是当前特异性药物的热门靶点。目前动物类冠状病毒的膜融合特异性治疗药物仍处于研发阶段, 而人源冠状病毒已有部分特异性药物用于临床。这类药物主要有肽类、小分子类和蛋白质类, 同时一些提高药物抑制效力的方法正在积极开发中。该综述介绍了针对冠状病毒膜融合过程中各个靶点不同类型的抑制剂, 并讨论了其优缺点及未来展望, 以期为冠状病毒的治疗和控制提供参考。

关键词 冠状病毒; 膜融合抑制剂; 肽; 小分子; 蛋白质

Advances in Inhibitors of Membrane Fusion of Coronavirus

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Abstract Coronaviruses are a type of enveloped RNA viruses that commonly infect humans and animals. They are known for their high transmissibility and pathogenicity, and can cause severe clinical symptoms and death. Developing specific medications is an effective strategy for controling coronavirus infection. The membrane fusion process plays a crucial role in the viral entry into target cells, and it is also a common target for specific medications. Drugs specifically targeting membrane fusion in animal coronaviruses are still in the research and development phase, while some drugs for human coronaviruses are already in clinical use. These medications primarily consist of peptides, small molecule and proteins, and some methods to improve the inhibitory effect of drugs are being actively developed. This review introduces various types of inhibitors that target different stages of the membrane fusion process in coronavirus, discussing their advantages, disadvantages and future potential to provide reference for the treatment and control of coronavirus.

Keywords coronavirus; membrane fusion inhibitor; peptide; small molecules; protein

冠状病毒种类繁多, 且常能引发人和动物的广泛性感染。在过去二十年里, 人类刚经历了包括严重急性呼吸系统综合征(severe acute respiratory syndrome, SARS)、中东呼吸综合征(Middle East respiratory syndrome, MERS)和新型冠状病毒肺炎(corona virus disease 2019, COVID-19)在内的三种严重的

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冠状病毒感染疫情。在动物中，鸡传染性支气管炎(infectious bronchitis virus, IBV)、猪delta冠状病毒(porcine deltacoronavirus, PDCoV)、猪流行性腹泻(porcine epidemic diarrhea, PED)和猪传染性胃肠炎病毒(transmissible gastroenteritis of swine, TGE)等也是常见的流行性疫病，严重威胁我国养殖业的健康发展。当前疫苗接种是我国防控冠状病毒感染的主要措施，但疫苗的免疫保护效果并不理想。特异性药物的研发是控制冠状病毒感染的有效途径。单克隆抗体、病毒复制抑制剂、膜融合抑制剂等都是该领域的研究热点。与前两者相比，病毒膜融合抑制剂还处于研发阶段，但其安全和生产成本较低，且能精确结合到目标蛋白的特点依然吸引研究者参与研究。本文将逐一介绍肽类、小分子类、蛋白质类冠状病毒膜融合抑制剂的特点，以及展望这三类药物的未来研究方向。

1 冠状病毒膜融合机制

冠状病毒基因组编码的病毒蛋白可分为结构蛋白、辅助蛋白和非结构蛋白，其中结构蛋白包括刺突蛋白(spike protein, S)、包膜蛋白(envelope protein, E)、膜蛋白(membrane protein, M)和核衣壳蛋白(nucleocapsid protein, N)。S蛋白是病毒复制过程中负责结合受体和介导膜融合的关键蛋白，由S1和S2亚基组成。S1亚基包括N-端结构域(N-terminal domain, NTD)和受体结合结构域(receptor binding domain, RBD); S2亚基包括融合肽(fusion peptide, FP)、七肽重复序列1(heptad repeat 1, HR1)结构域、七肽重复序列2(heptad repeat 2, HR2)结构域、跨膜结构域(transmembrane domain, TMD)和C末端的胞质区域(cytoplasmic domain, CD)(图1)。

首先，冠状病毒是通过S1亚基与宿主细胞上的特定受体结合进入细胞的，因此将S1亚基受体结合域RBD作为靶点，是开发膜融合抑制剂的一条途径[1]。其次，病毒可通过细胞表面直接融合和内吞两种途径进入靶细胞。病毒颗粒通过表面融合受体结合途径进入时，会引发S蛋白的构象变化。而通过内吞作用进入时，病毒颗粒结合受体后进入核内体，在低pH条件下触发S蛋白的构象变化，从而使S蛋白进入发夹前中间状态，进而使S2亚基的融合肽(FP)区域暴露并插入宿主细胞或核内体的膜中。在自然状态下，冠状病毒表面的S蛋白是无活性的。只有经过受体结合和蛋白酶水解后，S蛋白才会被激活，从而暴露S2亚基进行膜融合。多种宿主蛋白酶包括弗林蛋白酶(Furin)、跨膜丝氨酸蛋白酶2(transmembrane protease serine 2, TMPRSS2)和组织蛋白酶L都能作用于S蛋白，使其具有融合能力。因此，抑制这些宿主蛋白酶对S蛋白的水解也是开发

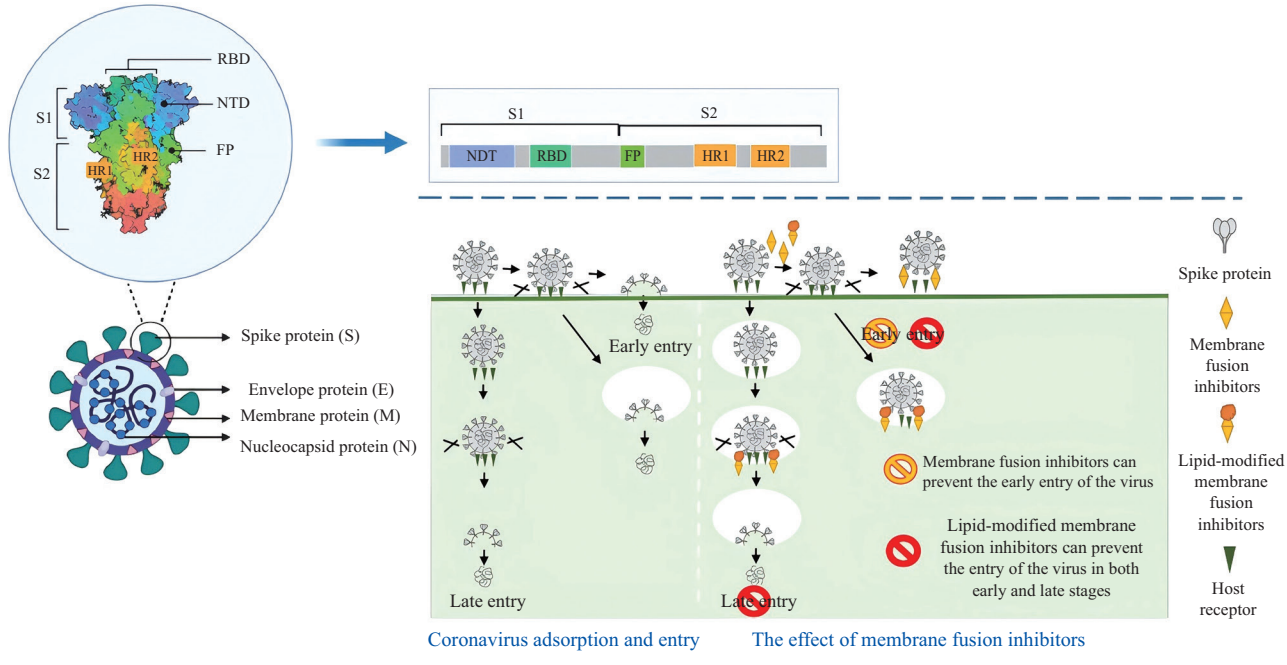


图1 冠状病毒S蛋白结构和膜融合抑制剂作用示意图

Fig.1 Schematic diagram of spike protein structure of coronavirus and effect of membrane fusion inhibitors

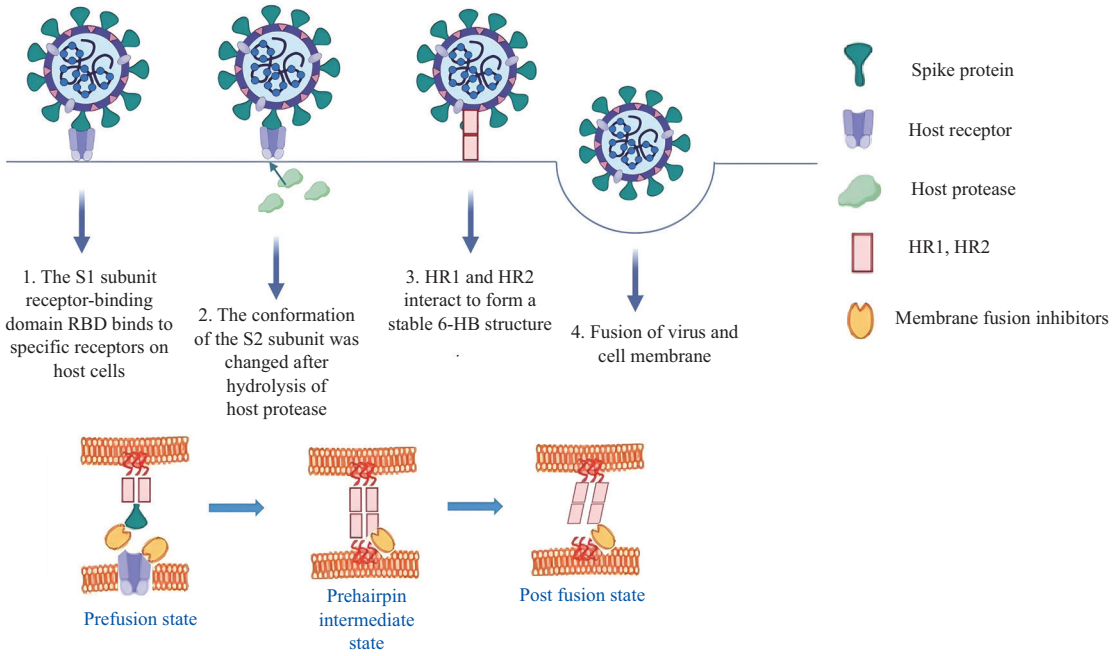


图2　冠状病毒膜融合机制和膜融合抑制剂作用靶点示意图

Fig.2　Schematic diagram of the mechanism of coronavirus membrane fusion and the target of membrane fusion inhibitors

广谱膜融合抑制剂的一条途径[2]。最后，当S1亚基经宿主蛋白酶水解，S2亚基暴露于细胞膜后，七肽重复区HR1与HR2相互作用，形成稳定的六螺旋束(six-helix bundle, 6-HB)，进而缩短病毒与细胞膜之间的距离，促进细胞与病毒的融合。因此，抑制HR1和HR2结合形成6-HB也是广谱冠状病毒膜融合抑制剂的重要研发方向[3](图2)。

2　肽类病毒膜融合抑制剂

2.1　靶向病毒S1亚基的RBD区域

冠状病毒S1亚基的RBD区域负责与细胞受体结合。因此，大量模拟病毒受体/共受体构象的肽可优先结合到冠状病毒的S1亚基上，从而阻断病毒与宿主受体结合进入靶细胞的过程。这些模拟病毒受体的特异性抑制剂除了在已有肽库中进行筛选外，还可以进行人工合成。例如，SARS-CoV和SARS-CoV-2的RBD可与宿主蛋白酶域的血管紧张素转换酶2(angiotensin-converting enzyme 2, ACE2)受体结合，部分研究者设想采用ACE2螺旋区的模拟肽作为诱饵受体，优先结合SARS-CoV-2，使病毒无法与宿主受体结合，从而阻止病毒感染。但最初设计的模拟线状肽NYBSP-C由于溶于液体后易失去其螺旋构象，因此在实验中显示对HT1080/ACE2和A549/ACE2细胞无抗病毒活性。随即研究者将这些小肽与碳氢化合物装订，增强了模拟肽的螺旋含量和对蛋白质水解的抵抗能力，并提高了其结合亲和力和生物活性，结果设计出的装订肽在实验中显示出对SARS-CoV-2的良好抑制活性[4]。模拟肽的设计为冠状病毒感染的抑制提供了一个可行思路，且人工修饰是提高其抑制效力的有效方法。

2.2　靶向病毒的HR1和HR2结构域

靶向病毒HR1或HR2的膜融合抑制剂可以阻断六螺旋束的形成，进而抑制膜融合。HR1和HR2都属于保守序列，且HR1的保守性优于HR2[5]。因此，理论上作用于保守HR1位点的膜融合抑制剂比靶向多变区的RBD、NTD中和抗体具有更广谱的冠状病毒抑制活性。在临床应用中，最早研究靶向HR1膜融合抑制剂的是针对人类免疫缺陷病毒(HIV-1)的恩夫韦迪(DP-178, T20)，随后多个针对HIV-1和冠状病毒等的肽抑制剂被研制[6]。尽管药物竞争性的与HR1或HR2结合均能够抑制病毒复制，但是HR1同源肽的抑制活性比HR2同源肽差，因为HR1肽具有高度疏水性，不能在水溶液中保持稳定的α螺旋结构，且易聚集于中性缓冲液中。目前，被成功证明能有效抑制冠状病毒复制的膜融合抑制剂均为靶向HR1肽的HR2同源肽，如抑制SARS-CoV的CP-1肽[7]，抑制MERS-CoV的MERS-HR2P和HR2P-M2肽[8]，抑制SARS-CoV-2的2019-nCoV-HR2P和IPB01肽[5,9]，以及

能抑制多种冠状病毒的EK1[5]、EK1C4[10]、OC43-HR2P[11]、SJ-2176 肽[12]、P3[13]等(表1)。尽管这些肽抑制剂多数仍处于临床前研究状态, 但其在保守性方面具有显著的研发优势。

裸露的HR2同源肽抑制剂存在易被消化、半衰期短、难以进入细胞内等问题。研究者为使该类抑制剂更好地应用于临床, 研究开发了许多增强HR2肽效力和延长其半衰期的方法。例如, 在西氟韦肽中引入E-K盐桥, 使HR2肽与HR1形成稳定的螺旋结构, 提高该类抑制剂对HIV-1的溶解度和亲和力[14]。在HR2肽的N-端和C-端添加MT钩和IDL锚使它们更易与HR1三聚体上的凹槽结合[15]。将脂质基团(如脂肪酸、胆固醇、鞘脂)与HR2肽连接, 使HR2肽凝结, 提高肽浓度。较高浓度的HR2肽与冠状病毒颗粒在宿主细胞表面充分融合, 显著延长了该类抑制剂的效力和半衰期[16]。另外, 结合白蛋白结合域或IgG Fc嵌合蛋白, 也被证明能有效延长HR2肽在体内的半衰期[17]。由于HR2区域通常在同一病毒属之间保守, 因此HR2肽抑制剂在属内也表现出广谱抑制的特点, 如HR1靶向肽EK1C4、[SARSHRC-PEG4]2chol、LCB1和LCB3在不影响宿主蛋白功能的情况下即可显示出对SARS-CoV-2的抗病毒效力[13,18](表1)。另外, 口服或吸入即可直接展现这些肽的活性, 而无需优化组织穿透性、血浆稳定性或半衰期等特性。另外, 药物在呼吸道中的降解将减少免疫反应和不良反应, 对于临床应用也是有益的。由此可见靶向冠状病毒HR1和HR2结构域的膜融合抑制剂具有较为理想的应用前景。

2.3 靶向宿主蛋白

病毒受体/共受体是具有生理功能的宿主蛋白和天然配体。因此, 天然配体本身, 或从这些受体/共受体的天然配体衍生出的肽, 也能够对病毒进入细胞起到抑制作用。研究发现一种防御素样肽P9R, 由于其拥有丰富的碱性氨基酸和对病毒糖蛋白高亲和力的特点, 因此其可以与病毒直接结合, 并对病毒–宿主内体酸化进行抑制。P9R可在体内外对多种pH依赖性呼吸道病毒如SARS-CoV-2、SARS-CoV、MERS-CoV表现出广谱抗病毒作用[19]。由于不同的病毒可能使用共同的宿主途径, 因此靶向宿主蛋白的抗病毒药物较靶向病毒融合蛋白药物更广泛有效。但这类抑制剂在临床使用中可能会干扰宿主的生理功能, 因此需要更全面地评价其安全性。

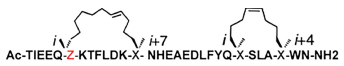
3 蛋白质类冠状病毒膜融合抑制剂

除了肽类外, 蛋白质也可作为冠状病毒S蛋白的抑制剂, 不同的是蛋白质类抑制剂往往在结构上模拟S蛋白的结合受体, 或是抗S蛋白的抗体, 质量和体积都较大。如SARS-CoV-2的受体ACE2相关蛋白衍生物在研究中显示出对SARS-CoV-2的有效抑制活性。然而, 重组可溶性ACE2衍生物要在相对较高浓度下才能抑制病毒感染, 因此研究者在ACE2的基础上设计了三聚体ACE2蛋白衍生物T-ACE2(表2), 该蛋白抑制剂对S蛋白具有极高的亲和力, 可以有效抑制包括SARS-CoV、SARSr-CoV、SARS-CoV-2和SARS-CoV-2的8种突变体在内的多种冠状病毒感染细胞[27]。另外, 重组ACE2-Ig融合蛋白对以ACE2为受体的假型SARS-CoV-2和SARS-CoV也均表现出中和活性, 且能抑制SARS-CoV-2和SARS-CoV S蛋白介导的细胞–细胞融合[28]。

冠状病毒抗S蛋白的抗体也可归类于蛋白质类膜融合抑制剂。这类具有抑制病毒膜融合功能的抗体有SARS-CoV中和抗体CR3022、m396和S109.8, MERS-CoV中和抗体m336和SAB-301, 以及SARS-CoV-2抗体31B5和32D4等, 但至今未报道有广谱抑制冠状病毒膜融合的抗体[29-33]。一些抗体在某些冠状病毒之间具有交叉反应性[34]。例如, SARS-CoV特异性单克隆抗体(mAb)、CR3022与SARS-CoV-2 RBD表现出交叉反应性结合; SARS恢复期患者血清在阻断SARS-CoV-2病毒进入方面也表现出交叉活性[35]。最近的一项研究报道了一种交叉中和的人抗体47D11, 它可以结合细胞上表达的全长刺突蛋白, 该抗体靶向RBD区域的一个保守表位, 对Vero E6细胞的SARS-CoV和SARS-CoV-2感染有抑制作用。47D11还能抑制SARS-CoV和SARS-CoV-2 S蛋白介导的细胞融合, 而对MERS-CoV-S蛋白介导的细胞融合无抑制作用[34]。现如今未发现MERS-CoV、SARS-CoV和SARS-CoV-2的交叉抗体。这表明与肽相比, 抗体在实现广谱冠状病毒交叉中和活性方面存在不足。另外, 冠状病毒抗S蛋白的抗体在动物冠状病毒中也有大量研究。例如, 能稳定分泌抗IBV S1蛋白抗体的杂交瘤细胞株4A11、1B11、5E5、7C9[36]和能够稳定分泌S2蛋白MAb的杂交瘤细胞株1A7、4F12、4A7、4D1等, 均对IBV显示出良好的抗病毒活性[37]; 还有能与PEDV S1蛋白产生特异性反应的单抗表达细胞株12D14H4、13F5B9、10D2B10、11A7C9和14E6F5, 也

表1 冠状病毒膜融合抑制剂(肽类)

Table 1 Coronavirus membrane fusion inhibitors (peptides)

药物 Drugs	靶向蛋白 Targeted proteins	序列或结构 Sequence or structure	作用机制 Mechanism of action	参考文献 References
NYBSP-4	S1 subunit of SARS-CoV and SARS-CoV-2		The structure that mimics the ACE2 helix region preferentially binds to the S protein receptors of SARS-CoV and SARS-CoV-2 in the hope of preventing the virus from binding to the host receptor	[4]
SP-10	S1 subunit of SARS-CoV	STSQKSIVAYTM	Blocking SARS-CoV S protein interaction with ACE2	[20]
P6	HR1 of SARS-CoV	GINASVVNIQKEIDRLNEVAKNL	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[21]
CP-1	HR1 of SARS-CoV	GINASVVNIQKEIDRLNEVAKNLNESL-IDLQELGKYE	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[7]
MERS-HR2P	HR1 of MERS-CoV	SLTQINTTLLDLTYEMLSLQQVVKAL-NESYIDLKEL	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[8]
HR2P-M2	HR1 of MERS-CoV	SLTQINTTLLDLEYEMKKLEEVVK-KLEESYIDLKEL	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[8]
MERS-5HB	HR1 of MERS-CoV	HR1-SGGRGG-HR2-GGSGGSGG-HR1-SGGRGG-HR2-GGSGGSGG- HR1	Inhibit the binding of HR1 and HR2 to form 6-HB	[22]
229E-HR1P	S2 subunit of HCoV-229E	AASFNKAMTNIVDAFTGVN-DAITQTSQALQTVATALNKIQDVVN-QQGNSLNHLTSQ	Inhibit the binding of HR1 and HR2 to form 6-HB	[23]
229E-HR2P	S2 subunit of HCoV-229E	VVEQYNQTILNLTSEISTLENKSAEL-NYTVQKLTQTLIDNINSTLVDLKWL	Inhibit the binding of HR1 and HR2 to form 6-HB	[23]
2019-nCoV-HR2P	HR1 of SARS-CoV-2	DIGINASVVNIQKEIDRLNEVAKNL-NESLIDLQEL	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[8]
IPB02	HR1 of SARS-CoV-2	ISGINASVVNIQKEIDRLNEVAKNL-NESLIDLQELK (cholesterol)	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[9]
EK1C4	HR1 of SARS-CoV-2, HCoV-OC43, HCoV- 229E and HCoV-NL63	SLDQINVTFLDLEYEMKKLEEAIK-KLEESYIDLKEL-GSGSG-PEG4-C (cho-lesterol)	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[10]
HR2-8	HR1 of SARS-CoV	ELDSFKEELDKYFKNHTSPDVDLGDIS-GINASVVNIQKEIDRLNEVAK	Inhibit the binding of HR1 and HR2 to form 6-HB	[10]
HR1-A	HR1 of SARS-CoV	YENQKQIANQFNKAISQIQESLTTTSTA	It is a truncated peptide of HR1 to inhibit the formation of 6-HB	[24]
GST-removed-HR2	HR1 of SARS-CoV	DVDLGDISGINASVVNIQKEIDRLNE-VAKNLNESLIDLQELGKYEQYI	It is a truncated peptide of HR2 to inhibit the formation of 6-HB	[24]
HR2P	HR1 of MERS-CoV	SLTQINTTLLDLTYEMLSLQQVVKAL-NESYIDLKEL	It is a derived peptides of HR2 to inhibit the formation of 6-HB	[8]
OC43-HR2P	HR1 of α-HCoV and β-HCoV	SLDYINVTFLDLQDEMNTLENKSAEL-NYTVQRLQEAIKVLNQSYINLKDI	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[11]
SJ-2176	HR1 of multiple coronaviruses	EWDREINNYTSLIHSLIEESQN-QQEKNEQEGGC	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[12]

续表1

药物 Drugs	靶向蛋白 Targeted proteins	序列或结构 Sequence or structure	作用机制 Mechanism of action	参考文献 References
[SARSHRC-PEG4] 2chol	HR1 of SARS-CoV-2	[DISGINASWNIQKEIDRLNEVAKNL-NESLIDLQEL -PEG4] 2-chol	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[13]
LCB1	RBD of the S1 subunit of SARS-CoV-2	DKEWILQKIYEIMRLLDEL-GHAEASMRVSDLIYEFMKKG-DERLLEEAERLLEEVER	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[25]
LCB3	RBD of the S1 subunit of SARS-CoV-2	NDDELHMLMTDLVYEALHFAKDEEIK-KRVFQLFELADKAYKNNDRQKLEKV-VEELKELLERLLS	It is an HR2 homologous peptide, which binds to HR1 and prevents the HR2 from forming a 6-HB structure with HR1	[26]
P9R	Host proteins	NGAICWGPCPTAFRQIGNCGRFRVRC-CRIR	It binds directly to the virus and inhibits virus-host endosomal acidification, thereby preventing the virus from infecting the host	[19]

被证实对PEDV有较高的抗体效价[38]; 另外杂交瘤细胞株2C2, 也被鉴定能特异性识别细胞中的TGEV, 且具有良好的抗体效价[39]。

另一组抑制剂不直接结合病毒S蛋白, 而是通过调节病毒受体表达来起到抑制病毒与宿主受体结合的作用, 如选择性雌激素受体调节剂下调ACE2表达, 也同样能有效抑制SARS-CoV、SARS-CoV-2等冠状病毒的进入[40]。

4 小分子类病毒膜融合抑制剂

小分子类药物, 由于其具有生产成本低、大多数能用于口服、临床利用度高的特点, 近几年也是病毒膜融合抑制剂的研究热点。研究中常用小分子来模拟多肽或蛋白质药物的功能。

4.1 靶向冠状病毒的S蛋白

冠状病毒S蛋白是近几年小分子类膜融合抑制剂研发的重要靶点, 如新型ACE2抑制剂*N*-(2-氨乙基)-1甲苯酰胺能抑制宿主ACE2受体的活性, 并能在微摩尔范围内阻断SARS-CoV S蛋白与ACE2受体的结合, 从而阻断病毒的膜融合过程(表3)。然而, 它只对以ACE2为受体的冠状病毒有效, 因此很难实现抑制剂的广谱性[43]。干扰素诱导跨膜蛋白(interferon-inducing transmembrane protein, IFITM)可以通过促进内体中胆固醇的积累来抑制多种包膜病毒进入宿主细胞, 如可以抑制MERS-CoV、SARS-CoV、HCoV-229E和HCoV-NL63的进入[44], 但IFITM不是冠状病毒的特异性抑制剂。因此, 阻断受体结合的小分子还需进一步研究。

4.2 靶向宿主蛋白

当前宿主的TMPRSS2、组织蛋白酶L常作为研发冠状病毒抑制剂的靶标蛋白。如蛋白酶抑制剂能够阻止SARS-CoV-2、SARS-CoV、MERS-CoV、HCoV-229E和HCoV-NL63等病毒感染靶细胞。目前, 对蛋白酶抑制剂的研究主要集中在小分子上, 且部分药物已经进行临床应用, 如甲磺酸卡莫司他和甲磺酸那莫司他均可以抑制TMPRSS2的活性, 以此阻断SARS-CoV-2进入人呼吸道上皮细胞。此外, 甲磺酸卡莫司他也对SARS-CoV和MERS-CoV S蛋白介导的膜融合有抑制作用。在药效方面, 研究发现甲磺酸那莫司他对SARS-CoV-2的抑制活性约为甲磺酸卡莫司他的15倍[45-46]。除TMPRSS2抑制剂外, 小分子化合物的组织蛋白酶L抑制剂K11777还可抑制假型SARS-CoV、MERS-CoV、HCoV-229E和HCoV-NL63的感染, 并可阻断SARS-CoV在Vero 76细胞中的复制。但K11777仅能在缺乏丝氨酸蛋白酶激活的细胞中发挥完全抑制病毒进入细胞的能力, 否则K11777需与丝氨酸蛋白酶抑制剂(甲磺酸卡莫司他、甲磺酸那莫司他)联合使用才能发挥药效。在P3位点修饰的K11777的衍生物SMDC256159和SMDC256160与K11777具有相似的体外抗病毒作用。但在SARS-CoV致死性感染小鼠模型中, 单次使用SMDC256160无明显保护作用。这些蛋白酶抑制剂的体内效率目前尚未报道。另一种组织蛋白酶L抑制剂SID-26681509在浓度为2 μmol/L时可抑制76%的SARS-CoV-2假病毒粒子进入293/hACE2细胞[47]。替考拉宁是一种糖肽类抗生素, 其氨基结构域含有疏水性基团, 使得其可抑制组织蛋白酶L的活性, 从而也能阻止假型MERS-CoV、SARS-CoV、SARS-CoV-2的感染。其他糖肽类抗生素, 如达巴万

表2 冠状病毒膜融合抑制剂(蛋白质类)

Table 2 Coronavirus membrane fusion inhibitors (proteins)

药物 Drugs	靶向蛋白 Targeted protein	作用机制 Mechanism of action	参考文献 References
T-ACE2	S1 subunit of the virus	Mimic the host's ACE2 receptor and preferentially bind to the viral receptor to prevent the virus from binding to the host receptor	[27]
Recombinant ACE2-Ig	S1 subunit of the virus	Mimic the host's ACE2 receptor and preferentially bind to the viral receptor to prevent the virus from binding to the host receptor	[28]
47D11	RBD of the S1 subunit of SARS-CoV-2	A conserved epitope targeting the RBD region, binding to the S1 protein	[34]
mAb	RBD of the S1 subunit of SARS-CoV	TargetRBD and bind to the S1 protein	[35]
m396	RBD of the S1 subunit of SARS-CoV	Target RBD and bind to the S1 protein	[29]
S109.8	RBD of the S1 subunit of SARS-CoV	Target RBD and bind to the S1 protein	[30]
m336	RBD of the S1 subunit of MERS-CoV	Target RBD and bind to the S1 protein	[31]
SAB-301	RBD of the S1 subunit of MERS-CoV	Target RBD and bind to the S1 protein	[32]
REGN3051	RBD of the S1 subunit of MERS-CoV	Target RBD and bind to the S1 protein	[41]
REGN3048	RBD of the S1 subunit of MERS-CoV	Target RBD and bind to the S1 protein	[41]
311mab-31B5	S1 subunit of the virus	Target RBD and bind to the S1 protein	[33]
311mab-32D4	S1 subunit of the virus	Target RBD and bind to the S1 protein	[33]
COVA1-18	RBD of the S1 subunit of SARS-CoV-2	Target RBD and bind to the S1 protein	[42]

星、奥利万星和特拉万星也表现出相同抑制活性，但缺乏疏水基团的万古霉素无法阻断SARS-CoV-2等冠状病毒进入靶细胞[48]。此外，广谱半胱氨酸蛋白酶抑制剂E64D在浓度为30 μmol/L时可抑制92.5%的SARS-CoV-2假病毒粒子进入细胞[49]。以上研究表明，小分子类冠状病毒膜融合抑制剂在研发和应用上都较其他类抑制剂成熟，是当前较为理想的特异性治疗药物。

5 总结

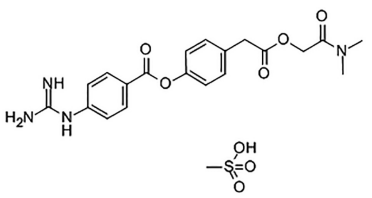
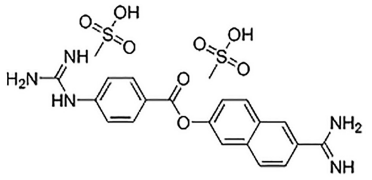
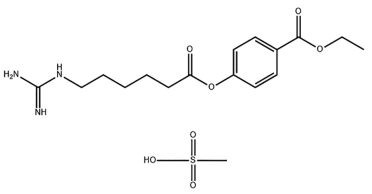
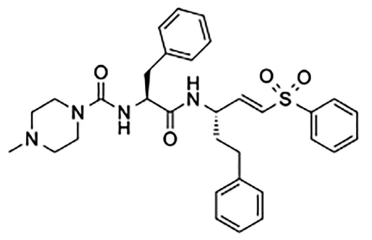
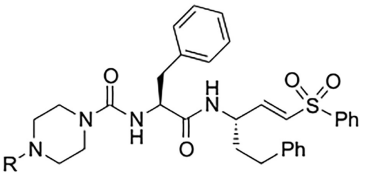
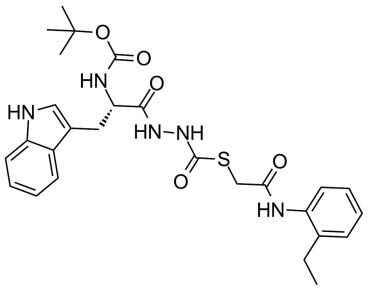
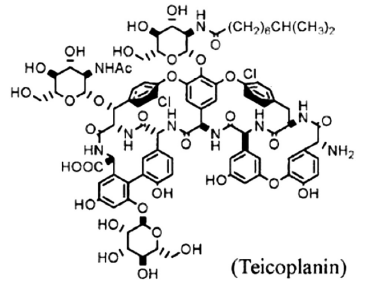
冠状病毒膜融合是病毒感染宿主的关键步骤。病毒S1和S2亚基是研究最多的抑制靶点。由于病毒S2亚基比S1保守，因此是研发广谱抑制剂的理想靶点。然而，由于病毒S1亚基暴露在病毒表面，而S2需要在S1亚基消化后才能暴露出作用位点。因此，能够中和游离病毒粒子和感染细胞的抗S1亚基抗体是当前应用最为广泛的特异性抑制剂。构建由S1和S2亚基两个靶点组成的双特异性蛋白质或抗体可以综合两者的优点，这类蛋白质也成为冠状病毒膜融合抑制剂未来一大研究方向。此外，包括S蛋白病毒受体/辅助受体、宿主蛋白酶和内吞作用调节剂在内的宿主蛋白对病毒进入细胞也至关重要，这类宿主蛋白的抑制剂往往具有广谱性的特点，可以靶向抑制多种病毒。然而，这些宿主蛋白的生理功能可能被抑制剂激活或破坏，产生治疗效果以外的不良影响。以S1亚基RBD作为膜融合抑制剂的靶点，由于RBD区域在不同冠状病毒之间的保守性较低，因此它可以成为特异性抑制剂的理想靶点，但不是广谱抑制剂的理想靶点。以S2亚基6-HB为靶点，破坏6-HB形成的肽抑制剂对不同的冠状病毒表现出有效的抑制活性，但需要考虑延长其半衰期和提高其口服利用度的问题。

对于膜融合抑制剂的形式而言，多肽通常含有3~50个氨基酸，其质量体积比小分子大，比蛋白质小，可以精确地结合到目标蛋白质上，从而产生更高的效力，其副作用也会较蛋白质更少。另外，多肽的代谢物是氨基酸，通常较为安全。与蛋白质相比，肽的尺寸较小，降低了它们诱导宿主对外来病原体免疫反应的潜力。较短长度的肽也有利于化学合成或进行原核表达，这可以节省药物生产的成本和时间。但是，肽的不稳定性是开发肽类药物的主要困难。多肽很容易被胃蛋白酶或其他宿主蛋白酶消化成氨基酸，从而缩短药物的血浆半衰期，因此很难口服用药，这大大减少了临床实用性。现如今，许多方法(包括氨基酸修饰和功能片段的偶联)已应用于提高肽类药物的效力和广度。

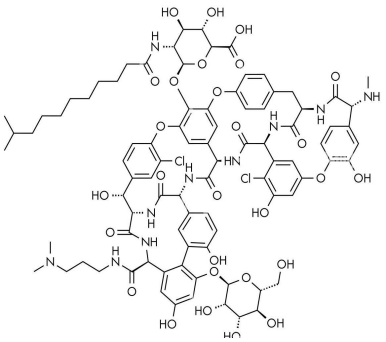
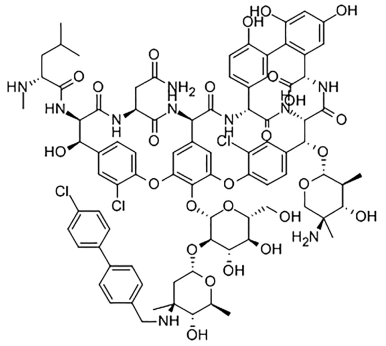
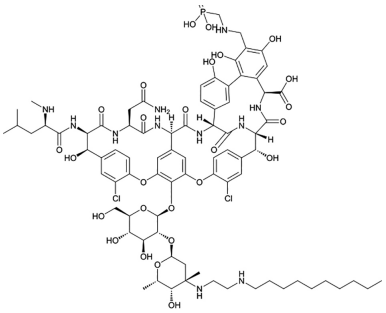
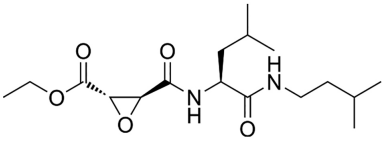
与多肽或小分子化合物相比，蛋白质类病毒膜融合抑制剂体积更大，它们可以以更高的特异性与目标蛋白质结合，不仅降低了副作用的风险，且在体内半衰期相对较长，增加了临床实用性。但这同时

表3 冠状病毒膜融合抑制剂(小分子类)

Table 3 Coronavirus membrane fusion inhibitors (small molecules)

药物 Drugs	靶向蛋白 Targeted protein	结构 Structure	作用机制 Mechanism of action	参考文献 References
Camostat mesilate	Host protease		Inhibit the activity of the TMPRSS2, thereby inhibiting coronavirus membrane fusion	[45]
Nafamostat (NM)	Host protease		Inhibit the activity of the TMPRSS2, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[45]
Gabexate mesilate (FOY)	Host protease		Inhibit the activity of the TMPRSS2, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[46]
K11777	Host protease		Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[47]
SMDC256159	Host protease	 R= —CH2CH2OCH3	Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[47]
SID-26681509	Host protease		Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[47]
Teicoplanin	Host protease	 (Teicoplanin)	Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[48]

续表3

药物 Drugs	靶向蛋白 Targeted protein	结构 Structure	作用机制 Mechanism of action	参考文献 References
Dalbavancin	Host protease		Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[48]
Oritavancin	Host protease		Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[48]
Telavancin	Host protease		Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[48]
E64D	Host protease		Inhibit the activity of the cathepsin L, thereby preventing the activation of the S protein and inhibiting the fusion of the coronavirus membrane	[49]

也增加了诱导药物特异性宿主免疫反应的风险。而且，由于宿主胃蛋白酶或其他宿主蛋白酶会消化蛋白质，也限制了蛋白质类药物的肠内使用。在临床研究中，进一步延长蛋白质类膜融合抑制剂的半衰期，进而减少用药频率，这成为近几年蛋白质类抗病毒药物的主要发展趋势。

小分子类膜融合抑制剂由于其具有易于生产和运输方便、价格便宜，而且不会被宿主体内蛋白酶降解，因此更适合口服给药的特点，被广泛用于临床研究。但是由于其质量较小，在宿主体内的半衰期也相对较短，比肽类和蛋白质类膜融合抑制剂的特异性差。小分子类膜融合抑制剂的研发依赖于化合物文库的大规模筛选，不同的实验室通常针对不同的病毒有不同的筛选系统。为了应对未来可能突然出现新型的冠状病毒，最好建立一个快速、稳定、大规模的系统。筛选出的先导化合物也需要进一步进行结构–活性关系分析修饰，以提高其效价和其他药代动力学特性。由于大多数临床药物都是小分子类的，重新利用已批准的药物可能是一种更快的方法来鉴别已有小分子药物是否能对新出现的病毒产

生抑制作用。然而重新利用的药物具有的多功能性也增加了副作用的风险。因此，重新利用药物治疗新发病毒的有效性和安全性也需要在标准化的临床试验中进行综合评价。

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